Evidence for Involvement of the Epidermal Platelet-Activating Factor Receptor in Ultraviolet-B-Radiation-Induced Interleukin-8 Production

Nicholas B. Countryman,*† Yong Pei,* Qiaofang Yi,* Dan F. Spandau,*‡ and Jeffrey B. Travers*†‡§

Departments of *Dermatology, †Pharmacology and Toxicology, ‡Biochemistry and Molecular Biology, §Pediatrics and the H.B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana, U.S.A.

Ultraviolet B radiation has been shown to generate cutaneous inflammation in part through inducing oxidative stress and cytokine production in human keratinocytes. Amongst the proinflammatory cytokines synthesized in response to ultraviolet B radiation is the potent chemoattractant interleukin-8. Though the lipid mediator platelet-activating factor (PAF) is synthesized in response to oxidative stress, and keratinocytes express PAF receptors linked to cytokine biosynthesis, it is not known whether PAF is involved in ultraviolet-B-induced epidermal cell cytokine production. These studies examined the role of the PAF system in ultraviolet-B-induced epidermal cell interleukin-8 biosynthesis using a novel model system created by retroviral-mediated transduction of the PAF-receptor-negative human epidermal cell line KB with the human PAF receptor. Treatment of PAF-receptor-expressing KB cells with the metabolically stable PAF receptor agonist carbamoyl-PAF resulted in increased interleukin-8 mRNA and protein, indicating that activation of the epidermal PAF receptor was linked to interleukin-8 production. Ultraviolet B irradiation of PAF-receptor-expressing KB cells resulted in significant increases in both interleukin-8 mRNA and protein in comparison to ultraviolet-B-treated control KB cells. Pretreatment with PAF receptor antagonists inhibited both carbamoyl-PAF-induced and ultraviolet-B-induced interleukin-8 production in the PAF-receptor-positive cells, but not in control KB cells. Similarly, treatment of the PAF-receptor-expressing primary cultures of human keratinocytes or the human epidermal cell line A-431 with carbamoyl-PAF or ultraviolet B radiation resulted in interleukin-8 production that was partially inhibited by PAF receptor antagonists. These studies suggest that the epidermal PAF receptor may be a pharmacologic target for ultraviolet B radiation in skin and thus may act to augment ultraviolet-B-mediated production of cytokines such as interleukin-8. ***Key words: IL-8/keratinocytes/oxidative stress/platelet-activating factor/UVB. J Invest Dermatol 115:267–272, 2000***

Ultraviolet B radiation (280–320 nm; UVB) can have profound effects upon human keratinocytes. Acute short-term UVB absorption by keratinocytes results in oxidative stress and DNA damage (Norins, 1962; Stewart *et al*, 1996). UVB can also induce cytokine production in keratinocytes including tumor necrosis factor α (TNF-α) and interleukin 1 (IL-1), IL-6, IL-8, and IL-10 (Kock *et al*, 1990; Kondo *et al*, 1993; Ullrich, 1995). Keratinocyte cytokine production has been implicated in both the proinflammatory and the immunosuppressive effects ascribed to UVB (Luger *et al*, 1997). Through its ability to act as a powerful neutrophil chemoattractant, IL-8 can have proinflammatory effects (reviewed by Harada *et al*, 1996). Resting keratinocytes do not synthesize appreciable amounts of IL-8, but will do so in the presence of other cytokines including IL-1α, IL-1β, TNF-α, or phorbol esters. Keratinocytes also express functional receptors for IL-8, and IL-8 has been reported to induce keratinocyte proliferation *in vitro* (Tuschil *et al*, 1992).

In addition to its ability to induce the production of protein cytokines, UV radiation can induce the production of lipid mediators such as prostanoids and platelet-activating factor (1-alkyl-2-acetyl-glycero-3-phosphocholine; PAF) in epidermal cells (Pentland *et al*, 1990; Sheng and Birkle, 1995; Barber *et al*, 1998). Derived from glycerophosphocholines, PAF is a potent activator of many cell types, including platelets, monocytes, polymorphonuclear leukocytes, mast cells, and vascular endothelium (reviewed by Pinckard *et al*, 1994). PAF also has trophic effects on diverse cell types (Roth *et al*, 1996). Although this glycerophosphocholine-derived mediator can be metabolized to potentially biologically active neutral lipid or phosphatidic acid species (Travers *et al*, 1990; Bauldry *et al*, 1991), the majority of PAF effects are thought to be mediated through a single G protein-linked transmembrane receptor (PAF-R) (reviewed by Izumi and Shimizu, 1995). The PAF-R is linked to numerous signal transduction pathways including PLA2, PLC, PLD, mitogen-activated protein kinase, tyrosine kinase, and adenylate cyclase. The ability of a single receptor to activate numerous signal transduction cascades explains the myriad of effects ascribed to PAF. Though PAF is the best

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Reprint requests to: Dr. Jeffrey B. Travers, H.B Wells Center for Pediatric Research, James Whitcomb Riley Hospital for Children Rm 2659, Indiana University School of Medicine, 702 Barnhill Drive, Indianapolis, IN 46202. Email: jtravers@iupui.edu

Abbreviations: CPAF; 1-hexadecyl-2-N-methylcarbamoyl-glycerophosphocholine; PAF, platelet-activating factor.

characterized ligand for the PAF-R, other natural products can bind to and signal through this receptor. These other ligands for the PAF-R include oxidized phospholipids derived from low-density lipoproteins (Smiley *et al*, 1991; Heery *et al*, 1995), lipopolysaccharide and protein A (Nakamura *et al*, 1992), lipotechoic acid moieties on *Streptococcus* species (Cundell *et al*, 1995), and 1-acyl-2-acetyl glycerophosphocholines (Triggiani *et al*, 1991; Travers *et al*, 1998). This diversity of ligands recognized by the PAF-R could potentially allow involvement of this system in a wide range of pathologic conditions including oxidative damage and bacterial infection.

Recent studies suggest that the PAF system is involved in keratinocyte function and skin inflammation. PAF is found in association with inflammatory skin diseases (Mallet and Cunningham, 1985; Travers *et al*, 1998), intradermal injections of PAF induce inflammation (Archer *et al*, 1984; Hellewell and Williams, 1989), and human keratinocytes both synthesize PAF and 1-acyl PAF species and express functional PAF-Rs (Michel *et al*, 1990; Travers *et al*, 1995, 1996). Activation of the epidermal PAF-R leads to the production of PAF, prostaglandins, IL-6, IL-8, the inducible form of cyclooxygenase (COX-2), and reactive oxygen species (Pei *et al*, 1998; Goldman *et al*, 1999).

It is not known at present whether the PAF system participates in UVB-induced cytokine production. UV radiation has been reported to be a stimulus for PAF biosynthesis in corneal epithelial cells, however (Sheng and Birkle, 1995). In addition, cytokines and the oxidative stress generated in response to UVB irradiation in epidermal cells can cause PAF production in other cell types (Camussi *et al*, 1987; Lewis *et al*, 1988). Recent studies from our group indicate that UVB can trigger the production of a lipid-soluble PAF-R agonistic activity that mass spectrometry studies reveal is not structurally PAF nor 1-acyl PAF species (Barber *et al*, 1998; Dy *et al*, 1999).

The objective of these studies was to assess whether PAF-R activation can modulate UVB-induced IL-8 production. Using a model system that our laboratory has developed by retroviral-mediated gene transduction to express the human PAF-R in the PAF-R-negative human epidermoid cell line KB (Pei *et al*, 1998; Travers *et al*, 1998), as well as PAF-R-positive primary cultures of human keratinocytes and the human epidermal carcinoma cell line A-431 (Travers *et al*, 1995), we present evidence indicating that the epidermal PAF-R can modulate UVB-induced IL-8 production.

METHODS

Reagents Routine chemicals, 1-alkyl-2-acetyl-3-glycerophosphocholine (PAF), 1-hexadexyl-2-N-acetyl-3-glycerophosphocholine (CPAF), and fatty-acid-free bovine serum albumin (BSA) were obtained from Sigma (St. Louis, MO). Growth media and supplements were purchased from Life Technologies (Gaithersburg, MD) and fetal bovine serum from Intergen (Purchase, NY). The PAF-R antagonists were kindly provided as follows: WEB-2086 from Boehringer Ingelheim (Ridgefield, CT), and A-85783 from Dr. James Summers, Abbot Pharmaceuticals (Abbott Park, IL).

Cell culture The PAF-R-positive epithelial cell line A-431 and PAF-R-negative KB were cultured as previously described (Travers *et al*, 1995, 1996). KB cells were transduced with the MSCV2.1 retrovirus containing the human leukocyte PAF-R cDNA as previously described (Pei *et al*, 1998; Travers *et al*, 1998). KB cell clones transduced with PAF-R (KBP) or with control MSCV2.1 retrovirus (KBM) were characterized by southern and northern blot analysis and by binding and calcium mobilization studies to demonstrate that the KB PAF-R was functional (Pei *et al*, 1998). All experiments were replicated with at least three different KBP or KBM clones. Primary cultures of human keratinocytes were obtained from neonatal foreskins as previously described (Kuhn *et al*, 1999) using Cascade media (Cascade Biologics, Portland, OR).

IL-8 measurements Epidermal cells were irradiated as previously described (Barber *et al*, 1998) using a Philips F20T12/UV-B source (270–390 nm; containing 2.6% UVC, 43.6% UVB, 53.8% UVA). The intensity of the UV source was routinely measured using an International Light radiometer equipped with a UVB detector.

Total RNA was extracted from KB cells and 20 μg RNA was subjected to northern blot analysis exactly as previously described (Pei *et al*, 1998). Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and IL-8 cDNA probes were obtained from American Type Culture Collection (Rockville, MD). IL-8 protein release was measured by enzyme-linked immunosorbent assay (ELISA) as previously described, with minor modifications (Pei *et al*, 1998). Briefly, KB cells were plated at 200,000 per ml on 24 well plates for 24 h, and then exposed to media with or without drugs and/or subjected to UVB irradiation. In experiments in which CPAF or phorbol myristate acetate (PMA) was used, cells were treated with CPAF or ethanol vehicle (0.1%). For experiments involving PAF antagonists, cells were preincubated with drug, ethanol, or dimethylsulfoxide (DMSO) vehicle (0.25%) for 30 min before UVB (400 J per m^2) treatment. The medium was collected at various times and IL-8 was assayed using Quantikine ELISA kits (R&D, Minneapolis, MN). Similarly treated cells were trypsinized and counted (Coulter). For the studies involving A-431 cells, the cells were plated at 100,000 per ml for 24 h. To decrease the spontaneous release of IL-8, cells were then placed in serum-free medium (with 0.1% BSA) for an additional 24 h before the experiment was conducted. Drugs were also placed in serum-free medium. Primary cultures of human keratinocytes were used within the first five passages, plated at 100,000 per ml for 48 h, and drugs were placed in the keratinocyte media.

Statistics Data are presented as the mean ± the standard deviation (SD). Statistical significance is assessed by the Student's *t* test and significance is set at $p < 0.05$.

RESULTS

The KB PAF-R model system As PAF may have both receptor-dependent and receptor-independent effects (secondary to the formation of biologically active metabolites), a model system was developed to study the role of the PAF-R in epidermal cell function. This system utilizes the human epidermal cell line KB, which, unlike normal human keratinocytes, does not express functional PAF-Rs (Travers *et al*, 1995, 1998). A PAF-R-positive (KBP) KB cell line was created by transducing KB cells with the replication-defective MSCV2.1 retrovirus containing the entire human PAF-R cDNA (Pei *et al*, 1998; Travers *et al*, 1998). By comparing the effects of stimuli on both KBP and PAF-R-negative (transduced with empty MSCV2.1 retrovirus; KBM) KB cells, the role of the PAF-R on IL-8 production could be readily assessed.

The effects of CPAF on IL-8 production in KB cells Our first studies assessed the ability of PAF-R activation to stimulate IL-8 production using the KB PAF-R model system. KBM and KBP cells were treated with 100 nM of the metabolically stable PAF-R agonist CPAF for various times and IL-8 mRNA or released protein was measured. As shown in **Fig 1**, incubation of KBP, but not KBM, cells with CPAF resulted in an increased accumulation of IL-8 mRNA. Similar to our previous studies (Pei *et al*, 1998), increased cytokine mRNA was first seen by 30 min, was maximal at 1–2 h, and returned to baseline by 6 h. Consistent with the northern blotting data, CPAF treatment of KBP cells resulted in an increase in immunoreactive IL-8 protein secretion, as shown in **Fig 2**. CPAF-induced IL-8 protein release was first seen by 2 h and was maximal by 8 h. CPAF treatment of KBM cells did not result in

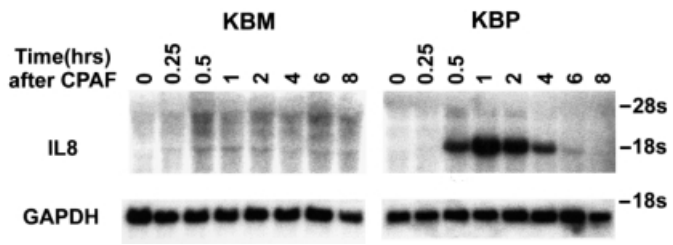


Figure 1. The effect of CPAF treatment on IL-8 mRNA levels in KB cells. KBP or KBM cells were incubated with 100 nM CPAF, and RNA was extracted at various times and subjected to northern blot analysis using probes for IL-8 or GAPDH. The results shown are typical for at least three separate KBM or KBP clones.

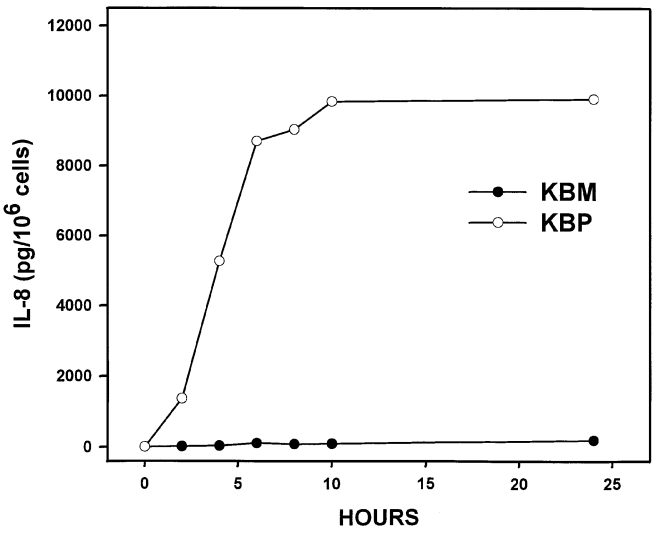


Figure 2. Measurement of IL-8 protein following treatment of KB cells with CPAF. KBP or KBM cells were incubated with 100 nM CPAF. The supernantants were removed at various times and assayed for immunoreactive IL-8 protein using a specific ELISA. The results shown are the mean ± SD of duplicate samples of a representative experiment of three conducted and are typical of at least three separate KBM or KBP cells. Statistically significant ($p < 0.05$) higher levels of IL-8 were measured in CPAF-treated KBP *versus* KBM cells at all time points.

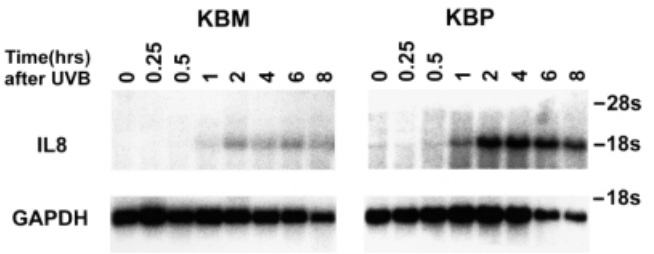


Figure 3. The effect of UVB treatment on IL-8 mRNA levels in KB cells. KBP or KBM cells were irradiated with 400 J per m^2 UVB, and RNA was extracted at various times and subjected to northern blot analysis using probes for IL-8 or GAPDH. The results shown are typical for at least three separate KBM or KBP clones.

a significant amount of IL-8 protein release. Treatment of KBM cells with 10 nM PMA, however, did result in increased IL-8 release (Pei *et al*, 1998), indicating that the PAF-R is not necessary for the production/release of this cytokine in these cells.

The effects of UVB on IL-8 production in KB cells Keratinocytes have been shown to synthesize IL-8 in response to UVB (Kondo *et al*, 1993). Given the findings that PAF-R activation is a potent stimulus for IL-8 production in epidermal cells, and UVB can induce a PAF-R agonistic activity in KB cells (Barber *et al*, 1998; Dy *et al*, 1999), the next experiments sought to define whether the presence of the PAF-R can modulate UVB-induced IL-8 biosynthesis. KBM and KBP cells were treated with 400 J per m^2 UVB and IL-8 mRNA or released protein was measured at various times following UVB irradiation. As shown in **Fig 3**, UVB irradiation of KBP cells resulted in a significant increase in the accumulation of IL-8 mRNA in comparison with similarly treated KBM cells. The time course of IL-8 mRNA accumulation in UVB-treated KBP cells appeared to be somewhat similar though of longer duration than that seen in CPAF-treated KBP cells with IL-8 mRNA still present at 8 h (compare **Figs 1, 3**). Consistent with the northern blotting data, UVB treatment of KBP cells resulted in increased IL-8 protein secretion in KBP over KBM cells (**Fig 4**). That UVB treatment of KBM cells did result in increased levels of IL-8 mRNA accumulation and protein release

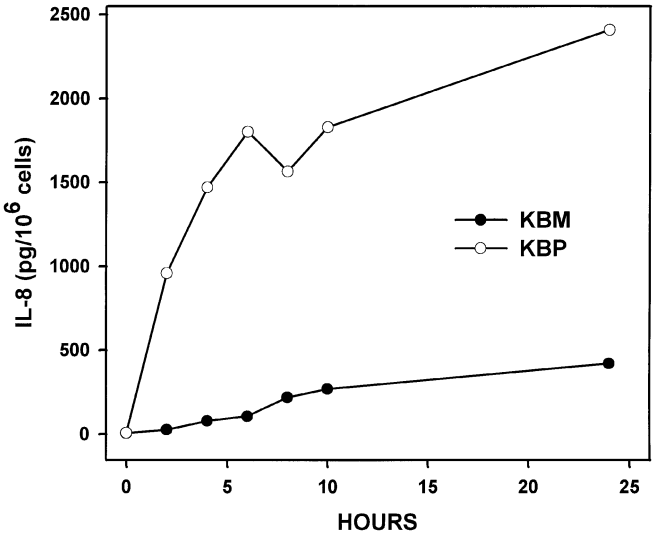


Figure 4. Measurement of IL-8 protein following treatment of KB cells with UVB. KBP or KBM cells were irradiated with 400 J per m^2 UVB, and the supernatants were removed at various times and assayed for immunoreactive IL-8. The results shown are the mean ± SD of duplicate samples of a representative experiment of three conducted and are typical of at least three separate KBM or KBP clones. Statistically significant ($p < 0.05$) higher levels of IL-8 were measured in UVB-treated KBP *versus* KBM cells at all time points.

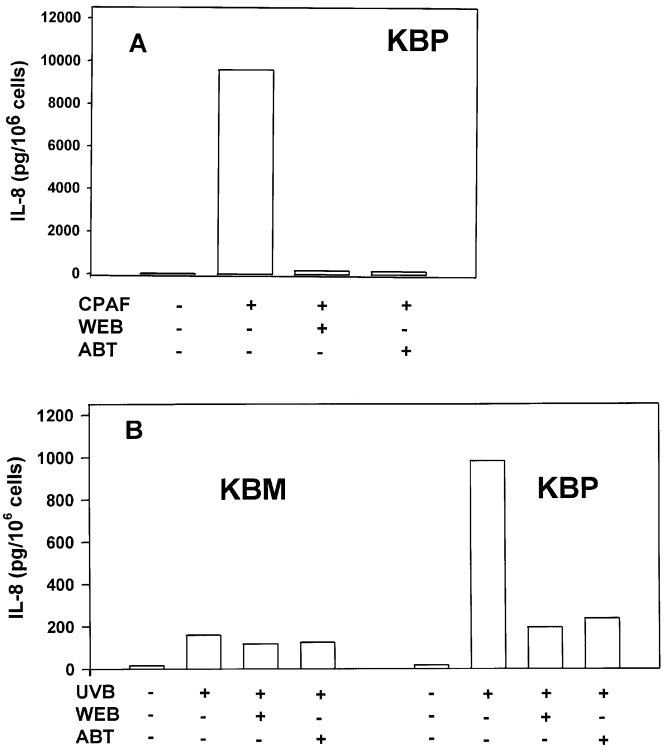


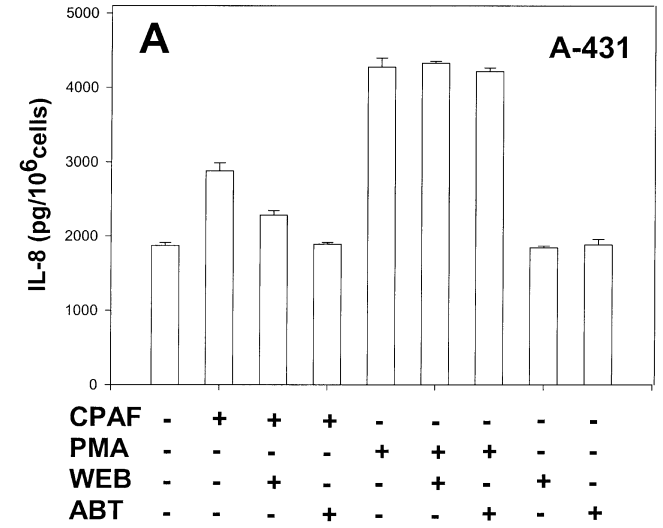
Figure 5. Effect of PAF-R antagonists on CPAF- and UVB-induced IL-8 release in KB cells. KBP or KBM cells were preincubated with 10 μM of the PAF-R antagonists WEB 2086 (WEB), A-85783 (ABT), or appropriate vehicle for 30 min prior to treatment with (*A*) 100 nM CPAF or (*B*) 400 J per m^2 UVB. Supernatants were removed at 8 h following UVB/CPAF treatment and assayed for immunoreactive IL-8. The results shown are mean ± SD of duplicate samples from a representative experiment of three conducted and are typical for two separate KBM and KBP clones.

(though at much lower amounts than in KBP cells) suggests that UVB can also act to increase IL-8 production independent of the epidermal PAF-R.

The ability of pretreatment with PAF-R antagonists to inhibit CPAF- and UVB-stimulated IL-8 release in KB cells was next assessed. As shown in **Fig 5(*A*)**, pretreatment of KBP cells with 10 μM of the PAF-R antagonists WEB 2086 or A-85783 (ABT) inhibited CPAF-induced IL-8 production. These PAF-R antagonists also inhibited UVB-induced IL-8 release in KBP cells but not in KBM cells (**Fig 5*B***). Of note, the levels of IL-8 measured in UVB-treated KBP cells pretreated with the PAF-R antagonists were similar to those in UVB-treated KBM cells. Incubation of KB cells with PAF-R antagonists did not affect baseline IL-8 release (**Fig 5*B***). These PAF-R antagonists do not affect IL-8 production induced by the phorbol ester PMA in KBP or KBM cells (Pei *et al*, 1998).

The effects of CPAF and UVB on IL-8 production in A-431 cells and primary cultures of human keratinocytes The next studies assessed whether the endogenous epidermal PAF-R could modulate IL-8 production as was found for the KB PAF-R. A-431 cells are a human epidermal-derived cell line that, like human keratinocytes, express functional PAF-Rs (Travers *et al*, 1995). As shown in **Fig 6(*A*)**, unstimulated A-431 cells were found to release significantly higher levels of IL-8 than KB cells (**Fig 2**) or primary cultures of human keratinocytes (**Fig 7**) (Kondo *et al*, 1993). Treatment of A-431 cells with 100 nM of the PAF-R agonist CPAF or 100 nM PMA resulted in increased IL-8 release (**Fig 6*A***). CPAF treatment resulted in less than a 2-fold increase in IL-8 production in A-431 cells, however, unlike the much greater increases seen following CPAF treatment of KBP cells. Preincubation of A-431 cells with the PAF-R antagonists WEB 2086 or A-85783 inhibited CPAF-induced but not PMA-induced IL-8 accumulation in these cells, indicating that CPAF exerted its effects through activation of the endogenous PAF-R. In addition, UVB irradiation of A-431 cells resulted in increased IL-8 release over baseline levels (**Fig 6*B***). As shown in **Fig 6(*B*)** for a typical experiment, pretreatment of A-431 cells with 10 μM of these two structurally dissimilar PAF-R antagonists decreased UVB-induced IL-8 production by approximately 20%–40%. Increasing the concentration of antagonists up to 25 μM did not result in further inhibition of UVB-induced IL-8 release (data not shown). Preincubation of A-431 cells with 1 μM of the PAF-R antagonist CV-6209 also inhibited UVB-induced IL-8 release similar to WEB-2086 and A-85783 (data not shown).

Treatment of primary cultures of human keratinocytes with CPAF also resulted in IL-8 accumulation (**Fig 7**). Pretreatment with PAF-R antagonists WEB 2086 (**Fig 7**) and A-85783 (not shown) inhibited CPAF-induced IL-8 release. Irradiation of human keratinocytes also resulted in IL-8 production. Though pretreatment of human keratinocytes with the PAF-R antagonists had inhibitory effects on UVB-induced IL-8 production, they were less dramatic than their inhibitory effects on A-431 or KBP cells. Altogether, these findings support the hypothesis that the epidermal PAF-R is not necessary for UVB-induced IL-8 production, yet the presence of this G-protein receptor can augment IL-8 production mediated by UVB.



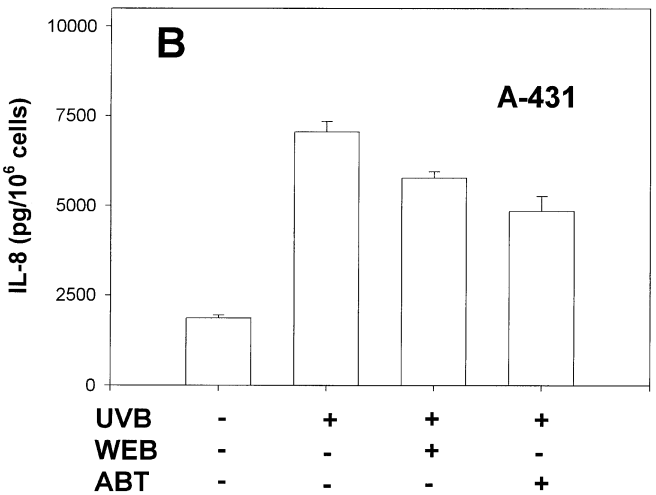


Figure 6. IL-8 production in A-431 cells. The PAF-R-positive epidermal cell line A-431 was treated with 10 μM of the PAF-R antagonists WEB-2086 (WEB) or A-85783 (ABT) or DMSO vehicle for 30 min before treatment with (*A*) 100 nM CPAF or 100 nM PMA, or (*B*) 400 J per m^2 UVB. Supernatants were removed at 8 h following CPAF/PMA/UVB treatment and assayed for immunoreactive IL-8. The results shown are mean ± SD of duplicate samples from a representative experiment of four to five conducted. Statistically significant ($p < 0.05$) increases in IL-8 release were seen following treatment with CPAF, PMA, or UVB. In addition, pretreatment with PAF-R antagonists resulted in statistically significant ($p < 0.05$) decreases in IL-8 release in response to CPAF and UVB, but not PMA.

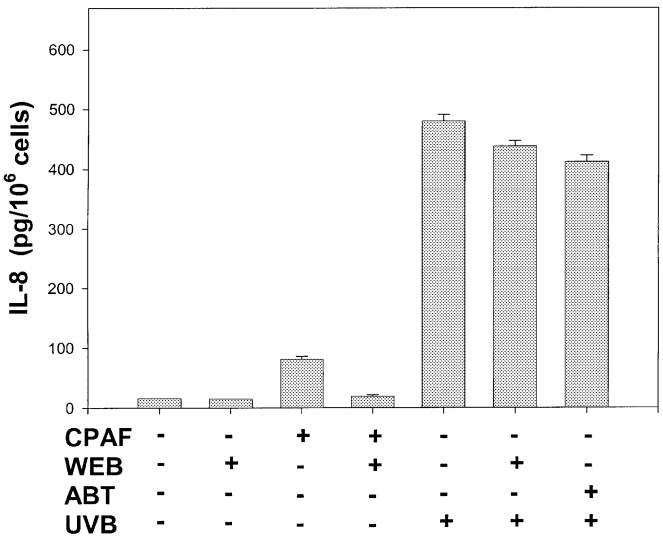


Figure 7. IL-8 production in primary cultures of human keratinocytes. The primary cultures of human keratinocytes were pretreated with 10 μM of the PAF-R antagonists WEB 2086 (WEB), A-85783 (ABT), or DMSO vehicle 30 min before treatment with 100 nM CPAF or 400 J per m^2 UVB, and IL-8 was measured 8 h later. The results shown are mean ± SD from a representative experiment of five conducted. Statistically significant ($p < 0.05$) increases in IL-8 were seen following treatment with CPAF and UVB. Pretreatment with PAF-R antagonists resulted in statistically significant ($p < 0.05$) decreases in IL-8 release in response to CPAF and UVB.

DISCUSSION

These studies provide evidence that the epidermal PAF-R may be a pharmacologic target for UVB, and thus involved in UVB-induced cytokine production. The study of PAF/PAF-R has been limited by the rapid metabolism of PAF and because PAF metabolites can exert biologic activity independent of the PAF-R (Wilcox *et al*, 1987; Travers *et al*, 1990). The model system used in these studies was developed to overcome some of the current limitations in the study of PAF/PAF-R, and to account for the diverse ligands recognized by the PAF-R (Smiley *et al*, 1991; Triggiani *et al*, 1991; Nakamura *et al*, 1992; Cundell *et al*, 1995). In particular, this model system can account for non-PAF PAF-R agonists such as *sn*-2 short-chain phosphocholines that have been shown to be produced in response to lipid peroxidation (Zimmerman *et al*, 1995; Murphy, 1996; Marathe *et al*, 1999). Interleukin-8, a member of the CXC family of chemokines, is an important activating stimulus and chemoattractant for neutrophils and thus has been implicated in many inflammatory diseases (Harada *et al*, 1996). The stimulus-specific production of IL-8 has been shown to be regulated primarily at the gene transcription level. Within the IL-8 promoter are found DNA binding sites for the inducible transcriptional factors NF-κB, NF-IL-6, and activation protein-1 (AP-1). Recent studies by Roebuck and colleagues indicate that these transcriptional factors can work in cooperative fashion to maximize IL-8 gene transcription (Lakshminarayanan *et al*, 1998; Roebuck *et al*, 1999). Of note, UVB can activate AP-1 and NF-κB pathways in human keratinocytes (Tobin *et al*, 1996; Chen *et al*, 1998). The PAF-R has also been shown to increase levels of these transcription factors in various cell types (Ye *et al*, 1996).

Activation of the KB PAF-R resulted in an increased accumulation of IL-8 mRNA and protein. Similarly, PAF treatment of various cell types from lung fibroblasts to neutrophils has also been reported to increase IL-8 biosynthesis (Bussolino *et al*, 1992; Roth *et al*, 1996). Inasmuch as IL-8 can also stimulate PAF biosynthesis in various cell types, including human keratinocytes (Pei *et al*, 1998), this lipid mediator and chemokine could be involved in the pathophysiologic effects ascribed to each other through positive feedback mechanisms. Characterization of this complex relationship between PAF and IL-8 could have therapeutic implications given the availability of PAF-R antagonists.

Consistent with the ability of UVB to generate PAF-R agonistic activity (Barber *et al*, 1998; Dy *et al*, 1999), UVB irradiation resulted in increased IL-8 mRNA accumulation and protein release in KBP over KBM cells. The kinetics of CPAF- and UVB-induced IL-8 mRNA accumulation differed as UVB-induced IL-8 mRNA accumulation was significantly longer (compare **Figs 1, 3**). These findings are compatible with UVB acting not only to stimulate the PAF-R but also to possibly affect IL-8 mRNA stability. Recent studies by Gilchrest and colleagues indicate that stabilization of mRNA is the primary mechanism by which UVB stimulates increased levels of TNF-α in keratinocytes (Leverkus *et al*, 1998). UVB-induced TNF-α production is also increased in KBP over KBM cells. Yet, UVB increases cyclooxygenase type 2 mRNA levels equally in KBM *versus* KBP cells (i.e., independent of PAF-R expression), suggesting different mechanisms for the induction of these various UVB-induced proteins (Dy *et al*, 1999). Future studies are planned to assess the functional cooperativity between transcriptional factors induced by the combination of UVB and PAF-R activation that results in maximal IL-8 production in epidermal cells.

In addition to the ability of UVB to generate PAF-R agonistic activity, several other possible mechanisms exist that could explain in part the ability of the PAF-R to augment UVB-induced IL-8 production. One possibility is that UVB could directly activate the PAF-R in a ligand-independent manner. Indeed, UV radiation has been reported to activate growth factor or TNF type 1 receptors directly (i.e., independent of ligand) (Rosette and Karin, 1996; Sheikh *et al*, 1998). Of note, the ability of UV to directly activate a G-protein membrane receptor has not been examined. The finding of a soluble PAF-R activity in UVB-irradiated KB cells, however, suggests that this activity is responsible for a component of UVB-induced PAF-R activation (Dy *et al*, 1999). We hypothesize oxidized lipids with PAF-R activity as the source of PAF-R-agonistic activity in KB cells. Recent studies have used mass spectrometry studies to identify 1-alkyl sn-2 short-chained fragmented glycerophosphocholines as the source of PAF-like lipids in oxidized low-density lipoprotein (Marathe *et al*, 1999). Future studies will attempt to structurally characterize this PAF-like activity, as well as to define whether UVB can activate a G-protein receptor directly.

Treatment of primary cultures of human keratinocytes and A-431 cells with the PAF agonist CPAF resulted in IL-8 accumulation, suggesting that the endogenous PAF-R and the expressed KB PAF-R are both linked to the production of this cytokine. PAF-R antagonists were less effective in UVB-induced IL-8 production, however, with 70%–80% inhibition in KBP, 20%–40% inhibition in A-431, and only 10%–20% inhibition seen in primary cultures of human keratinocytes. There are several explanations for what appears to be the lessening role of the PAF system in these three cell types. First, the numbers of PAF-Rs are much higher in KBP over the amounts seen in A-431, with even lower numbers of PAF-Rs in human keratinocytess in culture (Travers *et al*, 1995; Pei *et al*, 1998). The PAF-R in keratinocytes has been shown to use two separate promoters, however, one of which is inducible in response to cytokines including PAF and TNF through NF-κB activation (Izumi and Shimizu, 1995; Shimada *et al*, 1998). Upregulation of the human keratinocyte PAF-R in response to proinflammatory cytokines might result in more involvement of the PAF system in UVB-induced cytokine production. The relative lack of involvement of the PAF system in UVB-mediated IL-8 production in human keratinocytes in culture *versus* A-431 could also be due to the essential-fatty-acid-deficient culture conditions used to grow and expand human keratinocytes (Marcelo *et al*, 1992). The lack of essential fatty acids (such as arachidonic acid) would be significant as 1-alkyl *sn*-2 arachidonoyl glycerophosphocholines are the precursors of both PAF itself and oxidatively fragmented *sn*-2 species, which have been recently shown to be the biologic activity in oxidized low-density lipoproteins (Marathe *et al*, 1999).

Though human keratinocytes synthesize PAF (Michel *et al*, 1990; Travers *et al*, 1996) and express functional PAF-Rs (Travers *et al*, 1995), the role of the PAF-R in epidermal cell function is not clear. These studies suggest that one of the functions of the epidermal PAF-R could be to augment production of the known proinflammatory chemokine IL-8 in response to UVB radiation. These observations fit with the notion that the epidermal PAF-R may act as an endogenous ''damage sensor'', through its ability to recognize PAF and oxidatively modified glycerophosphocholines produced in response to noxious stimuli. Cytokines such as IL-8 or TNF-α produced by the epidermal PAF-R would then serve to draw blood-derived immune cells to the skin. Inasmuch as certain populations are potentially more susceptible to PAF effects due to inherited or acquired deficiencies in the enzymes involved in PAF degradation (PAF-acetylhydrolases) (reviewed by Stafforini *et al*, 1996), these findings may have clinical implications. A better understanding of the functions of the PAF system in keratinocyte biology and cutaneous inflammation may lead to therapeutic interventions designed around this lipid mediator.

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